EFFECTS OF TWO FUNGICIDES AND THREE ENVIRONMENTAL FACTORS ON THE UREDOSPORE GERMINATION OF *PUCCINIA ARACHIDIS* SPEG.

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ABSTRACT. – The process of germination of uredospores of *Puccinia arachidis* causing the rust of groundnut, *Arachis hypogaea*, was studied on glass slide, malt extract agar and host epidermal strip. The effects of spore concentration, light, temperature and relative humidity on spore germination of the fungus were also investigated. The emergence of germ tube occurred after 9 hrs, 12 hrs and 21 hrs on malt agar, host epidermis and glass slide respectively, while maximum germination was recorded after 18 hrs, 25 hrs and 35 hrs on the three substrates in the same order. Germination was highest on malt agar and least on glass slide. Optimum spore germination occurred on malt agar at 25°C, 89 % relative humidity, 600 Lux and spore concentration of 1.10^4 spores/ml. Benlate gave a better inhibitory effect than brestan when tried against the spore germination.

RÉSUMÉ. Étude du processus de germination des urédospores de *Puccinia arachidis*, provoquant la rouille des arachides : *Arachidis hypogaea*, sur lame de verre, sur milieu malt-agar et sur épiderme de l'hôte. Les effets de la concentration des spores, de la lumière, de la température et de l'humidité relative sur la germination des spores ont également été étudiés. Le tube germinatif apparaît après 9 h sur milieu malt-agar, 12 h sur épiderme de l'hôte et 21 h sur lame de verre; la germination maximale est enregistrée après 18 h, 25 h et 35 h respectivement sur les trois substrats pris dans le même ordre. La germination des spores est optimale sur milieu malt-agar à 25°C, 89 % d'humidité relative, 600 Lux et avec une concentration de 1.10^4 spores/ml. L'effet inhibiteur du Benlate contre la germination des spores est supérieur à celui du brestan.

KEY WORDS : Uredospore germination, *Puccinia arachidis*, fungicides.

INTRODUCTION

Puccinia arachidis Speg. is the causal organism of the rust of groundnut, *Arachis hypogaea* L. A review of literature shows that uredospores of rust fungi germinate less rapidly and less completely if they are removed from the

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sorus of uredium before being separated from their parent cells. COCHRANE (1960) pointed out that the overall rapidity of germination is a species characteristic: *Melampsora lini* (Pers.) Lev. begins germination in less than one hour but *Phoma apiicola* Klebahn spore requires 24-48 hours.

The effect of temperature on the germination of uredospores of *Puccinia* spp. indicates a wide range : *P. sorghi* Schw., 2-35°C (SHAW, 1964); *P. cynodontis*, 1.5-35°C (VARGAS & al., 1967). SUSSMAN & DONTHIT (1973) however, reported that low temperature depressed the germination of uredospores of *P. graminis* Pers. and those in cold dormancy germinated at 40°C when activated. the major effect of temperature is assumed to be on enzymatic process.

Day length and light intensity had little effect on the germination of *P. helianthi* Schw. but higher light intensity had an adverse effect on the germination of spores stored for two months at – 16°C (SOOD & SACKSTON, 1971, 1972). According to MADDISON & MANNERS (1973), nucleic acids and proteins of spores are affected by high light intensity.

The uredospore is the spreading phase of *Puccinia arachidis* which it actively produces on *Arachis hypogaea* throughout the growing season. Certain environmental factors therefore favour the existence of this stage of its life cycle. The control of this plant pathogen at this stage by protectant fungicides is in the problem of inhibiting the uredospore germination. The effects of two fungicides and some environmental factors on the process of uredospore germination of *P. arachidis* is being reported.

MATERIAL AND METHODS

The infected leaflets of *Arachis hypogaea* were collected from the Botanical nursery, Department of Botany and Microbiology, University of Ibadan, Nigeria. The preparation of the spore suspension was done by scraping uredospores from pustules on infected leaflets into 5 ml sterile distilled water. The suspension was then filtered through muslin cloth and the desired concentration was estimated using a Hawksley Cristallite B.S. 748 haemocytometer as described by PURVIS & al. (1966). A preliminary investigation on the effect of spore load on germination showed that 1.10^4 spores/ml was the concentration of spores that gave the most satisfactory results. Therefore except otherwise stated, this inoculum load was used throughout the study.

The experiments on spore germination on glass slide, 2 % malt extract agar and host epidermal strip were carried out. A drop of the spore load was placed on glass slide to form a film while another spore load was placed on each of four zones of Petri dish when two lines meeting at right angles were drawn at the bottom of plate. A teased epidermal strip from healthy host leaf was obtained, placed on a glass slide and a drop of spore load was inoculated on the strip. Each preparation was placed in a desiccator with about 90 % relative humidity at 28.5°C.

Variations in temperature were obtained in incubators set between 5-40°C, at 5°C interval, while the levels of relative humidity were obtained by using the method of WINSTON & BATES (1960). Solid phosphorus pentoxide and sterile distilled water gave 0 % and 100 % respectively. Four variations of light regimes were obtained : continuous darkness; continuous light; alternating light / darkness and alternating darkness / light. For the alternating periods of light and darkness, inoculated cultures were maintained 9 hours in continuous light and 9 hours in continuous darkness. For continuous darkness, Petri dishes containing the spores were wrapped with aluminium foil and kept in dark boxes. Light intensity was obtained by variations in degree of illumination with fluorescent bulbs and intensity produced was measured with a LI-COR photometer and expressed in Lux.

Water suspensions or solutions of brestan (fentin acetate or triphenyltin acetate, $C_{20}H_{18}O_2S$) and benlate (methyl-1-butyl carbamoyl-2 benzimidazole-carbamate) at 10, 50, 100, 250, 500 and 1000 ppm of active ingredients (DHANVANTARI, 1968) were prepared. One millilitre of the fungicide suspension/solution or sterile water (as control) was sprayed with a syringe on solidified surface of malt agar in Petri dishes, spread and allowed to stand for 4 hrs. One drop of spore suspension was then added to each of the four zones on medium, incubated at 28.5°C for 18 hrs. The spore germination was stopped at the required time with formalin acetic alcohol (5 : 5 : 90 v/v).

RESULTS

The uredospore measured between 15.7 - 22.9 µm at zero hour and between 20.0 - 30.0 µm prior before the emergence of germ tube. The process of uredospore germination showed the following : on the glass slide, emergence of germ tube occurred after 21 hrs of incubation and formation of hyphae occurred after 27 hrs; on malt agar, germ tube emergence was observed after 9 hrs while formation of hyphae occurred after 15 hrs; on host epidermal strip, after 12 hrs and 25 hrs of incubation, germ tube and hyphae were produced respectively. Maximum percentage germination of uredospores of 26 % at 35 hrs, 48 % at 18 hrs and 32.6 % at 25 hrs was recorded on glass slide, malt agar and epidermis respectively (Fig. 1).

The results obtained on the effect of spore concentration on germination showed that no germination was observed either at 100.10^4 spores/ml or 1000.10^4 spores/ml. The results further showed that poor appreciable germination were recorded at 10.10^4 spores/ml and at 1.10^4 spores/ml respectively (Table 1).

The results of the effect of temperature on germination revealed that good germination was recorded between 20-30°C, optimum at 25°C (Fig. 2) while germination was poor at 5°C and 35°C with no germination at 40°C.

Table 1 — Effect of spore concentration on the germination of uredospores of *Puccinia arachidis* on 2 % malt agar after 18 hrs of incubation at 28.5°C.

Tableau 1 Effet de la concentration des spores sur la germination des urédospores de *Puccinia arachidis* sur malt-agar 2 % après 18 hrs d'incubation à 28.5°C.

spore concentration (spores/ml)	total number of spores estimated	% mean germination
1.10^4	479.0	43.8 ± 1.8*
10.10^4	480.0	5.0 ± 0.1
100.10^4	478.0	0
1000.10^4	481.0	0

* standard error

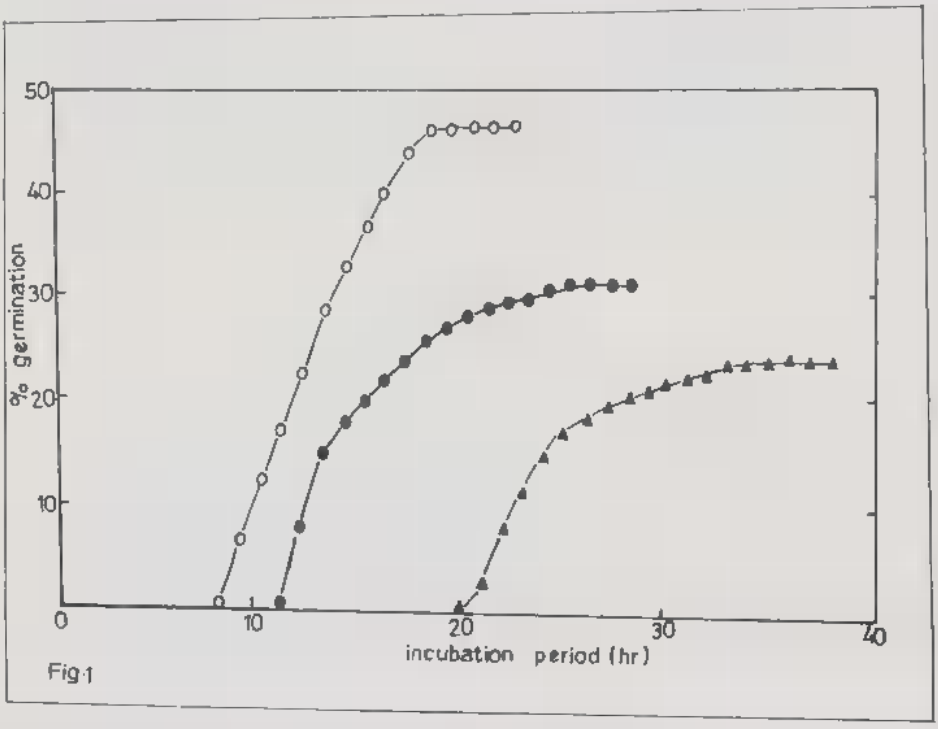

Figure 1 – Percentage germination of uredospores of *Puccinia arachidis* on three substrata : glass slide ▲–▲; host epidermal strip ● –●; malt agar ○–○; incubated for varying periods of time (hours) at 28.5°C under about 90 % relative humidity. Results are means of three replicates.

Figure 1 – Pourcentage de germination des urédospores de *Puccinia arachidis* sur les trois substrats : lame de verre ▲–▲; épiderme d'hôte ■–●; malt-agar ○–○; selon le temps d'incubation (heures) à 28.5°C et 90 % d'humidité relative environ. Les résultats sont les moyennes de trois expérimentations.

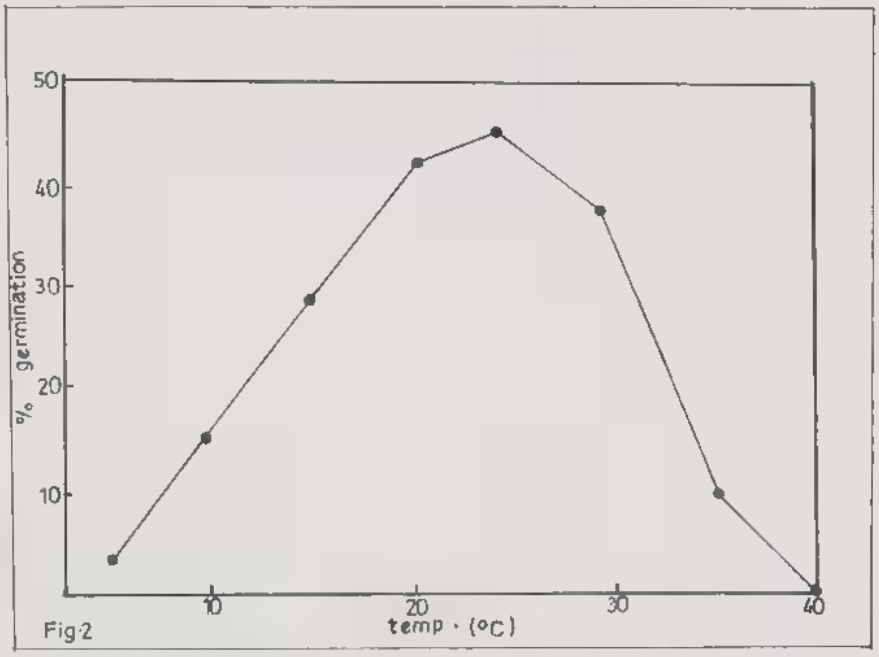

Figure 2 — Effect of temperature on the germination of uredospores of *Puccinia arachidis* on malt agar during 18 hrs of incubation. Results are means of three replicates.

Figure 2 — Effet de la température sur la germination des urédospores de *Puccinia arachidis* sur malt-agar pendant 18 heures d'incubation. Les résultats sont les moyennes de trois expérimentations.

The results of the effect of relative humidity levels on germination showed that no germination was recorded at 0 % while poor germination occurred at 32.5 % (Fig. 3). More uredospores germinated on malt agar than on glass slide, though each having optimum germination at 89 % level.

Table 2 — Effect of 4 light regimes on the germination of uredospores of *Puccinia arachidis* during 18 hrs of incubation at 28.5°C.

Tableau 2 — Effet de 4 régimes de lumière sur la germination des urédospores de *Puccinia arachidis* pendant 18 hrs d'incubation à 28.5°C.

light regimes	total number of spores estimated	% mean germination
continuous light	684.0	41.4 ± 2.3*
continuous darkness	664.0	47.4 ± 1.6
darkness/light (9 hrs each)	680.0	7.1 ± 0.3
light/darkness (9 hrs each)	660.0	10.1 ± 0.3

* standard error.

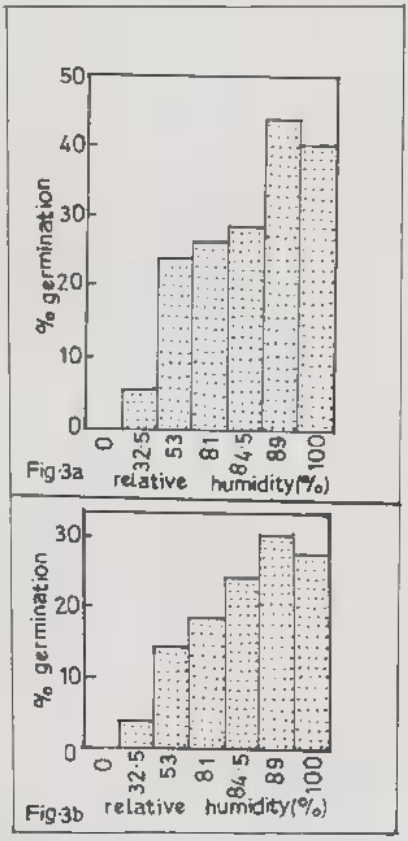

Figure 3 — Effect of relative humidity on the germination of uredospores of *Puccinia arachidis* on malt agar in 18 hrs (a) and on glass slide in 3 hrs (b) at 28.5°C. Results are means of three replicates.

Figure 3 — Effet de l'humidité relative sur la germination des urédospores de *Puccinia arachidis* sur malt-agar en 18 hrs (a) et sur lame de verre en 3 heures (b) à 28.5°C. Les résultats sont les moyennes de trois expérimentations.

Table 3 — Effect of brestan and benlate on the germination of uredospores of *Puccinia arachidis* after 18 hrs of incubation at 28.5°C.

Tableau 3 — Effet du brestan et du benlate sur la germination des urédospores de *Puccinia arachidis* après 18 heures d'incubation à 28.5°C.

fungicide conc. (ppm)	% mean germination recorded for each fungicide	
	brestan	benlate
0	29.2 ± 1.8*	25.8 ± 0.5
10	20.8 ± 1.6	9.5 ± 0.2
50	16.3 ± 1.1	5.0 ± 0.1
100	15.0 ± 1.0	2.5 ± 0.1
250	12.5 ± 0.7	0
500	5.7 ± 0.2	0
1000	1.0 ± 0.02	0

* standard error.

The results of the experiments on the effect of light regimes showed that poor germination was recorded when uredospores were exposed to alternating light and darkness and vice versa while good germination occurred when spores were under continuous exposure to either light or darkness (Table 2). As for the effect of light intensity on spore germination, no germination occurred at 2000 Lux while poor germination was recorded at 80 Lux and 1800 Lux. However, good germination was recorded at 140 Lux and 1500 Lux with the optimum at 600 Lux (Fig. 4).

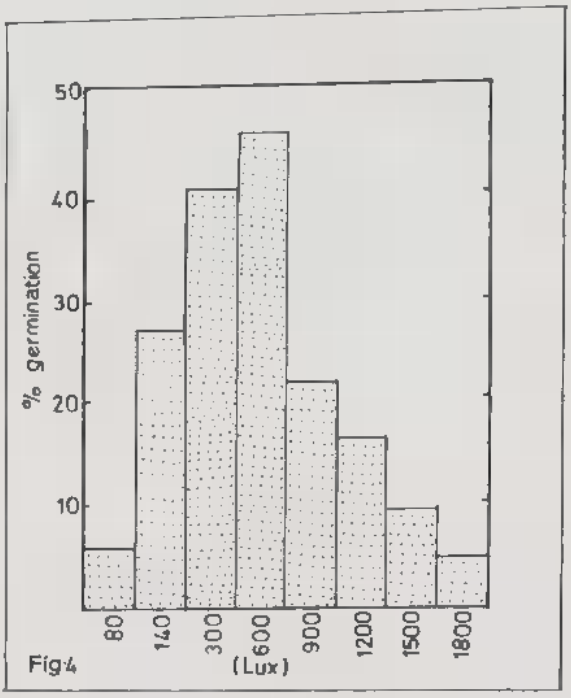

Figure 4 – Effect of light intensity on the germination of uredospores of *Puccinia arachidis* on malt agar in 18 hrs of incubation.

Figure 4 – Effet de l'intensité de la lumière sur la germination des urédospores de *Puccinia arachidis* sur malt-agar en 18 heures d'incubation.

The effect of the two fungicides on spore germination recorded 12.5 %, 5.7 % and 1.0 % at 250 ppm, 500 ppm and 1000 ppm respectively of brestan. With benlate, no germination was recorded at any of these three concentrations (Table 3).

DISCUSSION

Spores of lower moisture content absorb water to about 70 % before germination occurs (COCHRANE, 1958) and the frequent swelling of spores in water may be due to this dryness. The observations made on the swelling of uredospore prior to the emergence of germ tube indicated that the uredospores had absorbed water to certain considerable level and this may mean that uredospores of *P. arachidis* contained lower moisture.

The commencement of germination was fast on malt agar and epidermal strip (after 9 hrs and 12 hrs respectively) while it was delayed on glass slide (at 21 hrs). The findings of SHAW (1964), COCHRANE (1958) and SUSSMAN & DONTHIT (1973) showed that germination of spores takes longer period on non-absorptive medium like glass and that period of time is reduced by using solid substrate like agar which itself absorbs water from the atmosphere. The presence of nutrients in the medium and exudates on epidermal strip might possibly have stimulated the germination of the spores on these substrates. Such stimulating substances are absent on glass slide. However, the maximum germination (less than 50 %) recorded on each substrate was low. According to COCHRANE (1960), such an observation may be due to the immaturity of some of the uredospores.

There was no germination recorded at spore load of 100.10^4 and 1000.10^4 spores/ml. This may be due to self inhibition as a result of production of inhibitory substances (BROWN & HARVEY, 1927; GOTLIEB, 1950; ALLEN, 1955). On the other hand, that there was no germination at the two highest spore loads could also be due to competition for nutrients by these spores from their micro-environment. Germinating uredospores of *P. graminis* released volatile substances which inhibited germ tube elongation (ALLEN, 1955) while FORSYTH (1955) reported that uredospores of *P. graminis tritici* Erikss & Henn. produced a trimethylene compound (2 - methyl - butene - 2) which inhibited spore germination.

The uredospores germinated over a wide temperature range of 5-35°C with the optimum at 25°C. This range is similar to that obtained for *P. sorghi* by EVERSMERGER & BERLEIGH (1968). These observations may mean that inactivation of uredospores would occur at very cold and very high temperatures. The optimum percentage germination of the uredospores of *P. arachidis* occurred at 89 % relative humidity. This shows that this relative humidity would encourage more infections on the host. The following prevailing weather conditions : temperature of 26°-30°C; relative humidity of about 85 %; light intensity 347.2 Lux were recorded in the fields of the wetter South Western Nigeria where the disease was recorded. Furthermore, the fact that no germination was recorded at 0 % relative humidity and poor germination at 32.5 % relative humidity may be interpreted to mean that dry conditions would not favour the germination of the spores.

It is difficult to explain the poor germination of the uredospores as recorded in the alternating regimes of light even though alternating light and darkness

is the normal light regime in nature where the spore of the pathogen is exposed to. However, the ability of the uredospores to germinate on exposure to either light or darkness supported the earlier findings that light has no significant effect on germination as on sporulation (COCHRANE, 1960; DARBY & MANDELS, 1955). The results obtained on the effect of light intensity on germination was similar to that obtained for temperature. This observation could be due to the method used in obtaining variations in light intensity. Previous reports on light intensity on germination experiments tend to express the effect of illumination rather than degree of intensity. Variations of temperature occur with increase in light intensity.

Fungicidal action is a linear function of amount of toxicant taken up by spores. Brestan is highly toxic and fungicidal to about the same range of fungi as the copper fungicides but at about one-tenth the dosage (SPENCER, 1973). The mode of action of brestan is not widely reported however, we do speculate that it might act on the DNA or disrupt protein synthesis in the spores during germination. Benlate, a systemic fungicide, also shows from the literature that not much is known on its mode of action. Little is known about the ways in which systemic fungicides destroy or inactivate fungi (TARR, 1972). Because of the high toxicity of brestan and also because of the fact that benlate gave a better inhibitory effect on the germination of uredospores, benlate is therefore being recommended for field trials. These results obtained during this study would therefore facilitate a possible and effective control disease management technique on this spreading phase, the uredospore, of the pathogen. *P. arachidis* actively grows on the groundnut leaflets throughout the growing season.

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